

# Regulation of Fat Synthesis by Conjugated Linoleic Acid: Lactation and the Ruminant Model<sup>1–3</sup>

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## Abstract

Conjugated linoleic acid (CLA) isomers effect an impressive range of biological processes including the ability to inhibit milk fatty acid synthesis. Although this has been demonstrated in several mammals, research has been most extensive with dairy cows. The first isomer shown to affect milk fat synthesis during lactation was *trans*-10, *cis*-12 CLA, and its effects have been well characterized including dose-response relationships. Recent studies have tentatively identified 2 additional CLA isomers that regulate milk fat synthesis. Regulation by CLA occurs naturally in dairy cows when specific CLA isomers produced as intermediates in rumen biohydrogenation act to inhibit milk fat synthesis; this physiological example of nutritional genomics is referred to as diet-induced milk fat depression. Molecular mechanisms for the reduction in mammary lipid synthesis involve a coordinated down-regulation of mRNA expression for key lipogenic enzymes associated with the complementary pathways of milk fat synthesis. Results provide strong evidence of a role for sterol response element-binding protein 1 and Spot 14 in this translational regulation. Effects of CLA on body fat accretion have also been investigated in nonlactating animals, but CLA effects on mammary fatty acid synthesis occur at an order-of-magnitude lower dose and appear to involve very different mechanisms than those proposed for the antiobesity effects of CLA. Overall, results demonstrate the unique value of cows as a model to investigate the role of CLA in the regulation of milk fat synthesis during lactation. J. Nutr. 138: 403–409, 2008.

## Introduction

Nature has accorded lactation a high priority, and during lactation the extensive use of nutrients for the synthesis of milk imposes a substantial demand on the mother. Fat is the major energy source in milk, and lipid synthesis by the mammary glands is particularly impressive. In early lactation, daily milk fat secretion in the dairy cow can represent over 35% of net energy intake (1). Thus, Rudolph et al. (2) have aptly described the lactating mammary gland as “a lipid-synthesizing machine.”

Lactating ruminants have been of special value as a model to investigate milk fat synthesis. Knowledge of mammary uptake of nutrients, biosynthesis pathways, and the relation between diet and milk fat composition have been elaborated in studies with ruminants, and results have been extended and contrasted with those from other mammals (3,4). Although much is known about the biochemistry of milk lipid synthesis, the regulatory and cellular signaling systems of mammary fat synthesis are not well understood. The lactating bovine mammary gland synthesizes easily measurable quantities of lipid and thus is a unique model to study both the acute and chronic regulation of milk fat synthesis. When challenged, temporal changes in milk fat can be monitored in the same animal by sequential collection of milk samples and serial tissue biopsies. In contrast, quantifying milk yield, sequential collection of representative milk samples, and obtaining serial mammary biopsies are challenging in most other mammals. Similarly, investigations of lipid synthesis in nonlactating animal models generally require several weeks of treatment to quantify differences in adipose tissue accretion rates accurately, and multiple measurements are not typically obtained from the same animal over an experimental time course.

The discovery that certain conjugated linoleic acid (CLA)<sup>7</sup> isomers are potent inhibitors of mammary lipid synthesis in

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<sup>7</sup> Abbreviations used: CLA, conjugated linoleic acid; MFD, milk fat depression; S14, Spot 14; SREBP, sterol response element-binding protein.

dairy cows presents a unique opportunity to use this species as a model. CLA are produced during rumen fermentation, and their regulation of milk fat synthesis is a naturally occurring example of nutritional genomics in dairy cows that involves an interrelation between rumen digestive processes and mammary metabolism (4). In addition, CLA alter lipid metabolism and body fat accretion in nonlactating animals (5,6). Although the mode of regulation by CLA is not fully understood in either model, comparison of similarities and differences between its effects on mammary and adipose tissues provide additional mechanistic insight. In the following sections we address lactation responses to CLA, identify the specific CLA isomers that regulate milk fat synthesis, discuss recent investigations elaborating regulatory mechanisms, relate results to the low-fat milk syndrome in dairy cows, and, where appropriate, compare these findings to the reported antiobesity effects of CLA.

### CLA inhibits milk fat synthesis in dairy cows

CLA is a generic term used to describe positional and geometric isomers of octadecadienoic fatty acids with a conjugated double bond. Although CLA were first identified in milk fat in the 1930s (7), interest in CLA increased dramatically when CLA were identified as anticarcinogens and shown to have a wide range of potential health benefits in biomedical studies with animal models (8,9). Ruminant-derived food products are the primary source of dietary CLA for humans (10), and initial studies in lactating cows were designed to examine the transfer of exogenous CLA to milk fat for the purpose of creating a “functional food.” Unexpectedly, a dietary supplement containing a mixture of CLA isomers resulted in an immediate and dramatic reduction in milk fat secretion (11,12).

Most research investigating CLA effects during lactation has utilized dairy cows, and results have consistently demonstrated that the inhibitory effects are specific for milk fat; yields of milk and other milk components are generally unaffected (4). The reduction in milk fat secretion reaches a nadir by 4 to 5 d of supplementation and returns to previous levels in a similar temporal pattern when CLA treatment is terminated. Most studies have lasted a few days, but long-term studies (20 wk) indicate that the reduction in milk fat persists throughout the treatment period (13,14). Treatment has also encompassed all phases of the lactation cycle with no adverse effects on animal health and well-being (13–15). Interestingly, initial studies observed that CLA supplements were less effective at reducing milk fat during the first 3 wk postpartum (14,16). Subsequent studies have demonstrated that cows are responsive during this period, but a larger dose of CLA is required to achieve a similar reduction in milk fat yield (17,18).

### Relation to low-fat milk syndrome

The low-fat milk syndrome, more commonly referred to as milk fat depression (MFD), is a naturally occurring situation in dairy production when cows are fed highly fermentable diets or dietary supplements of plant or fish oils (4). First described over a century ago, diet-induced MFD can result in a reduction in milk fat yield of up to 50%, and the decrease involves fatty acids of all chain lengths. Effects are also specific for milk fat, as yields of milk and other milk components are generally unaffected.

Many theories have been proposed to explain the basis for diet-induced MFD, and most have proven inadequate (4,19). A key development was the demonstration that the reduction in milk fat was correlated with changes in the milk fatty acid profile, specifically an increased content of rumen-derived biohydrogenation intermediates. Referred to as the “biohydro-

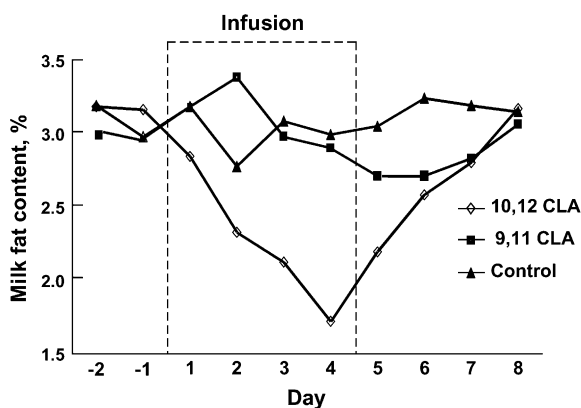
genation theory,” the basis for diet-induced MFD relates to an inhibition of mammary lipid synthesis by specific fatty acids that are intermediates in the biohydrogenation of dietary polyunsaturated fatty acids and are produced only under certain conditions of rumen fermentation (20). The first of these unique intermediates to be identified as a potent inhibitor of milk fat synthesis was *trans*-10, *cis*-12 CLA, and for many situations of diet-induced MFD, the increase in milk fat content of *trans*-10, *cis*-12 CLA is correlated with the magnitude of the reduction in milk fat yield (20,21).

In certain situations of diet-induced MFD, the *trans*-10, *cis*-12 CLA content and magnitude of the reduction in milk fat yield do not align with the dose-response curve generated with abomasal infusion of relatively pure *trans*-10, *cis*-12 CLA. This suggests that in these situations this single isomer does not completely explain the extent of the decrease in milk fat. Thus, additional inhibitory biohydrogenation intermediates have been proposed (20,22), and 2 additional isomers (*trans*-9, *cis*-11 and *cis*-10, *trans*-12 CLA) have recently been identified, as discussed in a later section. However, careful accounting of the rumen production of CLA isomers under different situations of diet-induced MFD indicates that the isomers identified to date are still not adequate to fully explain the observed decrease in milk fat yield (23). Nevertheless, diet-induced MFD is a natural situation in which, under certain dietary conditions, the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates that are potent inhibitors of milk fat synthesis.

### Regulation of milk fat synthesis is related to specific CLA isomers

CLA are produced as intermediates in the biohydrogenation of linoleic acid by rumen bacteria. *Cis*-9, *trans*-11 is the major CLA isomer in ruminant fat (~75–90% of total CLA), and it arises mainly from endogenous synthesis via  $\Delta^9$ -desaturase in the mammary gland; as the predominant CLA isomer in ruminant fat, *cis*-9, *trans*-11 is the CLA isomer with functional food implications (24). Milk fat, however, contains most of the other 24 possible CLA isomers, albeit each at a level generally 1% or less of total CLA. Studies in lactating and nonlactating animals have generally used a mixture of CLA isomers. We examined effects of relatively pure CLA isomers on milk fat synthesis and provided the isomers to dairy cows by abomasal infusion to avoid alterations by rumen bacteria (25). Results clearly demonstrated that *trans*-10, *cis*-12 CLA was responsible for the reduction in milk fat in dairy cows, and milk fat was rescued when treatment ceased (Fig. 1). In contrast, *cis*-9, *trans*-11 CLA had no effect on milk fat content or yield.

Milk fat content of many CLA isomers increases under dietary conditions of MFD (21,23), and the ability to investigate effects of specific isomers on milk fat synthesis has been limited by commercial availability. Laboratory synthesis of CLA isomers and/or combinations of CLA isomer enrichments, however, has allowed several others to be studied (Table 1). In addition to the well-established effects of *trans*-10, *cis*-12 CLA, *trans*-9, *cis*-11 CLA and *cis*-10, *trans*-12 CLA were identified as inhibitors of milk fat synthesis (26,27). Thus, a total of 3 CLA isomers have been identified as potent inhibitors of milk fat synthesis in dairy cows, although results with *trans*-9, *cis*-11 CLA and *cis*-10, *trans*-12 CLA should be interpreted cautiously because each involves a single study at a single dose. Nevertheless, compared with a similar dose of *trans*-10, *cis*-12 CLA, the *cis*-10, *trans*-12 isomer was equally or slightly more effective in reducing milk fat yield, whereas *trans*-9, *cis*-11 CLA was about one-half as potent.



**FIGURE 1** Temporal pattern of milk fat content during abomasal infusion of CLA supplements. Infusions were for 4 d, and treatments were: control, *cis*-9, *trans*-11 CLA (10 g/d), and *trans*-10, *cis*-12 CLA (10 g/d). Values are means,  $n = 3$ . Adapted from Baumgard et al. (25).

### Regulation of milk fat synthesis is dependent on CLA dose

De Veth et al. (31) combined results from 7 studies and indicated that abomasally infused *trans*-10, *cis*-12 CLA incorporation into milk fat was linear with a mean transfer efficiency of 22% (Fig. 2). Most importantly, a curvilinear relation was demonstrated between the increasing dose of *trans*-10, *cis*-12 CLA and the reduction in milk fat production. The median effective dose ( $ED_{50}$ ) corresponded to a 25% reduction in milk fat yield and occurred at a dose of 2.5 g/d of *trans*-10, *cis*-12 CLA ( $\sim 0.01\%$  of dry matter intake), and the maximum inhibition of milk fat secretion was  $\sim 50\%$ .

Results from dose-response studies clearly demonstrate that *trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis in dairy cows. As discussed in a later section, *trans*-10, *cis*-12 CLA also inhibits body fat accretion in growing animals, albeit at a substantially greater dietary dose. Bell and Kennelly (34) examined the effects of a mixed isomer preparation at a daily dose that provided 45 g of *trans*-10, *cis*-12 CLA via abomasal infusion. They observed that milk fat, as well as milk and milk lactose yields, were reduced and suggested that this pharmacological dose resulted in changes characteristic of involution (i.e., apoptosis) based on observed effects on somatic cell count

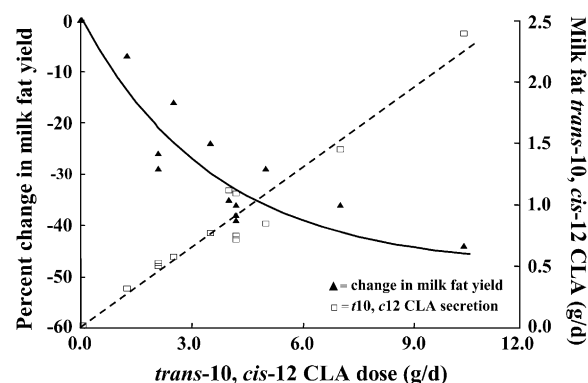
**TABLE 1** Typical concentration of investigated CLA isomers in bovine milk fat and their effect on milk fat yield when tested by abomasal infusion

CLA isomer tested	Total CLA isomers in milk fat, <sup>1</sup> %	Effects on milk fat yield	Reference
<i>trans</i> -8, <i>cis</i> -10	<0.1–1.5	NC <sup>2</sup>	(28)
<i>trans</i> -9, <i>cis</i> -11	<0.1–1.5	Inhibition <sup>3</sup>	(27)
<i>cis</i> -9, <i>trans</i> -11	72.6–91.2	NC	(25,29,30)
<i>trans</i> -9, <i>trans</i> -11	0.8–2.9	NC	(27)
<i>trans</i> -10, <i>cis</i> -12	<0.1–1.5	Inhibition	(25,27–33)
<i>cis</i> -10, <i>trans</i> -12	<0.1–1.5	Inhibition	(26,27)
<i>trans</i> -10, <i>trans</i> -12	0.3–1.3	NC	(26,27)
<i>cis</i> -11, <i>trans</i> -13	0.2–4.7	NC	(28)

<sup>1</sup> Adapted from summary by Lock and Bauman (24).

<sup>2</sup> NC = No change in milk fat yield when abomasally infused at a dose comparable to *trans*-10, *cis*-12 CLA.

<sup>3</sup> Inhibited milk fat yield when abomasally infused at a dose comparable to *trans*-10, *cis*-12 CLA.



**FIGURE 2** Relations between abomasally infused dose of *trans*-10, *cis*-12 CLA and 1) change in milk fat yield ( $y = -48.26 + 49.03\exp^{-0.2792x}$ ;  $R^2 = 0.86$ ) and 2) secretion of *trans*-10, *cis*-12 CLA into milk fat ( $y = 0.2175x + 0.0111$ ;  $R^2 = 0.94$ ). Adapted from a summary by de Veth et al. (31) based on data from (25,26,28,29, 31–33).

and chlorine content of the milk. The dose used by Bell and Kennelly (34) is almost 20-fold greater than the  $ED_{50}$  for the reduction in milk fat synthesis (Fig. 2) and approaches the dietary dose used in studies demonstrating the antiobesity effects of CLA in nonlactating animal models, as addressed in a later section.

### CLA regulates milk fat in other mammals

The ability of CLA to regulate milk fat synthesis has also been observed in other mammals including mice (35), rats (36,37), pigs (38,39), sheep (40,41), goats (42,43), and humans (44). Most of these investigations have used dietary supplements containing a mixture of CLA isomers. However, a study with rats demonstrated that milk fat content and nursing pup growth were reduced when *trans*-10, *cis*-12 CLA was provided as a dietary supplement, whereas *cis*-9, *trans*-11 CLA had no effect (35).

Variation in CLA dose, interval of administration, and response endpoint make it difficult to directly compare results from different lactating mammals to those from cows. Nevertheless, CLA supplementation has consistently resulted in a reduction in milk fat content, milk fat yield, and/or growth rate of the nursing neonate. Exceptions are studies with lactating women, where a reduction in milk fat content was observed in 1 study (44) and no effect was reported in another (45); interestingly, these 2 studies were conducted by the same group, and the basis for the difference in milk fat response to CLA supplementation is not obvious.

### CLA-induced milk fat reduction alters nutrient partitioning

Investigations of CLA in dairy cows have predominantly involved established lactation (i.e., post peak during galactopoiesis) when nutrient intake is adequate and energy balance is zero or positive. During this period, studies have consistently demonstrated that basal plasma concentrations of glucose and insulin were unaltered with *trans*-10, *cis*-12 CLA administration, although the expected reduction in milk fat yield was observed (13,14,29,46,47). Furthermore, *trans*-10, *cis*-12 CLA did not alter plasma glucose response to an insulin challenge (29,47). In addition, administration of *trans*-10, *cis*-12 CLA had little or no effect on basal plasma nonesterified fatty acid concentrations (13,15,29,47) and negligible effects on plasma

nonesterified fatty acid response to a  $\beta$ -adrenergic challenge (epinephrine) (29,47). Circulating leptin and insulin-like growth factor-I are related to energy homeostasis, and treatment with *trans*-10, *cis*-12 CLA had no effect on their plasma concentrations (25,29). Furthermore, CLA resulted in no unexpected changes in whole-body heat production (48) or indices of thermal load (49). Overall, results demonstrate that the CLA-induced reduction in milk fat yield occurs with no apparent alterations in glucose and energy homeostasis, thermal homeostasis, or whole-body bioenergetics.

Although most CLA supplementation trials have not detected an increase in either milk yield or component synthesis, experiments have typically been conducted while cows are in a positive energy and nutrient balance. There are, however, frequent situations when nutrient intake is insufficient to meet lactation demands (e.g., immediately postpartum and in pasture-based systems). Inducing a reduction in milk fat output with CLA supplements often results in an increase in the yields of milk and/or milk protein during early lactation (14,18,50) or with pasture systems and underfeeding models (46,47,50). A similar repartitioning effect was reported in sheep supplemented with *trans*-10, *cis*-12 CLA, resulting in an increase in milk and protein yields (40). Consequently, it appears that during times of inadequate nutrient intake, inducing a reduction in milk fat increases available energy that can be repartitioned toward the synthesis of milk or milk protein, and there are currently commercial CLA supplements available in some countries that are marketed for this specific purpose.

During periods when net energy intake is adequate, the CLA-induced reduction in milk fat yield coincides with marginal changes in feed intake (reduced) and body reserves (increased), although demonstrating statistical significance of these modest differences is difficult. The latter is best illustrated in bioenergetic studies where the 35% reduction in milk fat was accompanied by an increase in the energy retained in body tissues (48). Likewise, Harvatine et al. (51) demonstrated that the mRNA expression for lipogenic enzymes was increased in adipose tissue when CLA induced a reduction in milk fat of dairy cows. A repartitioning of energy to body fat reserves is also observed in diet-induced MFD (19).

### Mechanism involves a coordinated regulation of mammary lipid synthesis

Phenotypic characterization of CLA-induced MFD provides key insight into the functional mechanism of CLA. Fat is the only milk component inhibited with *trans*-10, *cis*-12 CLA treatment, and the reduction involves fatty acids of all chain lengths, thereby demonstrating that mammary effects are highly specific for lipid synthesis and include biochemical pathways associated with both de novo synthesis and the use of preformed fatty acids. Expression of lipogenic enzymes is coordinately stimulated by a class of transcription factors known as master regulators of lipid synthesis, and 1 of these is the sterol response element-binding protein (SREBP) family (52). Specifically, SREBP1c is highly responsive to changes in mammary lipid synthesis and up-regulated at the initiation of lactation in the mouse (2). Disruption of the SREBP1c gene results in a 41% decrease in milk fat concentration in mice (53), and this is strikingly similar to the maximum milk fat reduction (~50%) observed during *trans*-10, *cis*-12 CLA treatment (Fig. 2) or diet-induced MFD (4,31).

We first evaluated the SREBP-regulatory system in bovine mammary epithelial cells (MAC-T cell line) and observed decreased abundance of the nuclear SREBP1 protein (active fragment) during *trans*-10, *cis*-12 CLA inhibition of fatty acid

synthesis (54). SREBP1 is highly expressed in bovine mammary tissue, and recent investigations demonstrated that mammary expression of SREBP1 and proteins involved in the activation and translocation of SREBP were reduced for both *trans*-10, *cis*-12 CLA treatment and diet-induced MFD (55). Many lipogenic enzymes have SREBP response elements in their promoter, and, consistent with this, transcription of mammary genes involved in the complementary pathways for milk fat synthesis was coordinately down-regulated during CLA- and diet-induced MFD (Table 2). Collectively, these observations are consistent with SREBP1 representing a major signaling mechanism in the regulation of fatty acid synthesis during CLA-induced MFD. Polyunsaturated fatty acids, especially long-chain (n-3) fatty acids, are well recognized to inhibit lipid synthesis, and SREBP1 is 1 of the predominant signaling mechanisms in the regulation of hepatic fatty acid synthesis (56,57).

Mammalian regulation typically includes redundant systems for amplification of signaling and regulation of biochemical processes. Spot 14 (S14) is an SREBP1-responsive gene that encodes a nuclear protein that is closely associated with the regulation of fatty acid synthesis in lipogenic tissues including lactating mammary tissue (62). Although its exact biochemical function is not known, S14 has been implicated in the transcriptional regulation of lipogenic genes. Furthermore, S14 knockout mice have decreased milk fat concentration as a result of decreased de novo fatty acid synthesis, although surprisingly, activities of mammary lipogenic enzymes were unaltered (63). In lactating cows, mammary expression of S14 was down-regulated during diet-induced MFD and *trans*-10, *cis*-12 CLA treatment (55). Using publicly available microarray data from studies with mice, we also observed a significant reduction in expression of S14 in adipose tissue whose mass was decreased by CLA supplements (55). Thus, S14 may be more broadly implicated in the mechanism by which CLA affect lipid metabolism.

Some members of the nuclear hormone receptor family are known to bind and become activated by fatty acids, including CLA, and PPAR-centric mechanisms for the biological effects of CLA have been proposed for some tissues (64,65). To date, there

**TABLE 2** Summary of SREBP1-regulated lipogenic genes in bovine mammary tissue that demonstrate a coordinated reduction in expression during treatment with *trans*-10, *cis*-12 CLA or diet-induced MFD<sup>1</sup>

Biochemical process/enzymes	<i>trans</i> -10, <i>cis</i> -12 CLA Reference citations	Diet-induced MFD Reference citations
Synthesis de novo		
Acetyl-CoA carboxylase (EC 6.4.1.2)	(58,61)	(22,59,60)
Fatty acid synthase (EC 2.3.1.38)	(55,58,61)	(22,55,60)
Preformed fatty acids		
Lipoprotein lipase (EC 3.1.1.34)	(55,58)	(22,55)
Fatty acyl-CoA ligase (EC 6.2.1.3)		(22)
Desaturation		
$\Delta^9$ -Desaturase (EC 1.14.19.1)	(58, 61)	(22, 55, 60)
Esterification		
Acylglycerol phosphate acyltransferase (EC 2.3.1.51)	(58)	(22)
Glycerol phosphate acyltransferase (EC 2.3.1.15)	(58)	(22)

<sup>1</sup> Adapted from Harvatine and Bauman (55).



is no evidence of a role of PPAR in regulation of milk fat synthesis in the mammary gland or as a mechanism of CLA-induced MFD. However, in extramammary tissues where the PPAR family of transcription factors are highly expressed and are key regulators of tissue-specific differentiation and inflammation, they may be important in functional responses to higher doses of CLA.

Only a limited number of mechanisms have been investigated in CLA-induced MFD and these predominantly at the level of gene expression. The coordinated down-regulation of lipogenic enzymes during MFD is expected to involve multiple regulatory systems and the interaction of multiple signals. Mechanisms regulating lipid synthesis and SREBP1c continue to be identified and will provide strong hypotheses to test the regulation of milk fat synthesis. Specifically, AMP-activated protein kinase (66), protein kinase B (67), and extracellular signal-related kinase (68) signaling have not been investigated during MFD and represent logical mechanisms known to regulate lipid synthesis.

### Relation to antiobesity effects of CLA

Coinciding with initial studies examining CLA effects on milk fat synthesis was the discovery that dietary supplements of CLA, specifically *trans*-10, *cis*-12 CLA, caused a reduction in body fat accretion in mice (69,70). Subsequent studies extended these results to other animal models as well as humans (5,6,71,72). In general, there exists substantial inter- and intraspecies variation in the ability of CLA to induce body weight changes and reduce adipose tissue accretion among studies. This is in contrast to the relatively consistent results observed across lactation studies with *trans*-10, *cis*-12 CLA as discussed earlier. Reasons for these discrepancies are not entirely clear, but there are many examples of similarities and differences in lipid metabolism between lactation and growth.

Comparison among studies is difficult because the effect of CLA can be influenced by the CLA isomer mixture and dose as well as duration of treatment. The majority of studies have used a mixture of 2 or more isomers of CLA, although a few investigations have used relatively pure preparations of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. CLA isomers are known to differ in biological effects, but there is a consensus that the reduction in body fat is caused by the *trans*-10, *cis*-12 isomer (5,6). The amount of CLA necessary to reduce milk fat synthesis (<0.01–0.05% of diet) is very low (Fig. 2) compared with the amount of CLA reported to reduce body fat accretion (0.5–2.0% of diet). In contrast to the situation for milk fat synthesis, there are few well-defined dose-response curves for effects on body fat. Dose relations have been established only in the growing pig where the reduction in fat accretion was found to be linearly related to dose (dose range = 0.1–1.0% dietary intake) (72,73) and humans where meta-analysis indicated a linear relation between the reduction in body fat mass and the increase in CLA dose (dose range = 1–7 g/d) (71). However, establishing a dose-dependent relation involving adipose tissue accretion is more difficult because the endpoint is based on cumulative effects, whereas daily milk fat secretion can be quantified routinely.

Numerous theories have been advanced to explain the effects of *trans*-10, *cis*-12 CLA on lipid metabolism in adipose tissue, and some of the major ones include 1) increased basal metabolic rate and energy expenditure, 2) increased mobilization and oxidation of fatty acids from adipose tissue, 3) reduced preadipocyte proliferation and/or differentiation, and 4) increased adipocyte apoptosis (5,6,8). None of these corresponds to the effects of CLA on milk fat synthesis. The absence of effects on bioenergetics and heat expenditure and glucose and energy

homeostasis during the CLA-induced reduction in milk fat was discussed previously. Likewise, effects related to decreases in proliferation/differentiation or increases in apoptosis are not part of the response in the CLA-induced reduction in milk fat; of the milk components synthesized by the mammary gland, fat is the only one that is affected by CLA treatment of cows, and this is reversed when CLA treatment is terminated.

The CLA-induced reduction in adipose tissue has also been correlated with adverse side effects in some situations, including insulin resistance and fatty liver (6,74–76). Insulin resistance is unlikely to play a role in the reduction of milk fat synthesis because ruminant mammary tissue, in contrast to adipose tissue, is largely refractory to changes in circulating insulin (4). As discussed earlier, plasma insulin levels are unchanged, and glucose response to an insulin challenge is unaltered, in lactating cows treated with *trans*-10, *cis*-12 CLA. Furthermore, liver lipid content is not altered in cows receiving CLA treatment (14,15). Consequently, supplementing CLA to lactating dairy cows, either to enhance the CLA content in milk or to improve whole-animal energetics during specific stages of lactation, has no apparent negative effects on animal health and well-being. Furthermore, the level of *trans*-10, *cis*-12 CLA observed in milk fat when CLA supplements are used (31) is several orders of magnitude less than that needed to supply the CLA levels used in antiobesity studies (71).

### Final comments

Ruminant synthesis of milk fat has proven a unique model that has provided novel mechanistic insight in the regulation of fatty acid synthesis by CLA. This article highlights new discoveries but also raises additional challenging questions relating to fatty acid biosynthesis. For example: Are there other rumen biohydrogenation intermediates that regulate milk fat synthesis, and what is structurally/biologically different about the CLA isomers that do? Compared with adipocytes, what is the basis for the greater sensitivity of mammary epithelial cells to the CLA-induced inhibition of lipid synthesis? Following CLA uptake by mammary cells, what events are upstream of the SREBP response? To what extent are other fatty acid sensor proteins, cellular signaling factors, and molecular mechanisms involved in the lipogenic response to CLA? These questions serve to illustrate that regulation of fatty acid synthesis by CLA will remain an exciting research area, and the lactating ruminant will continue to be a valuable model to address scientific questions in this area.

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